IMMUNOLOGICAL DETECTION OF RecA-RELATED PROTEINS IN BULL MEIOTIC NUCLEI

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Using antibodies raised to recA protein purified from E.coli we have seen staining of distinct structures in unfixed isolated nuclei at different meiotic stages. The meiotic nuclei were isolated from bull testis [1]. The isolated nuclei were attached to freshly cleaved mica [2] and used unfixed for indirect staining with FITC-conjugated secondary antibodies, before or after extraction and digestion. It was important to use the unfixed nuclei as we recognized that any kind of fixation abolished or diminished remarkably the staining specificity of nuclear structures. Nuclei at pachytene revealed uniform staining of fine meiotic chromosome cores, that we suggest to be the synaptonemal complexes or their components. This suggestion is supported by the observation that the delicate green-stained cores were surrounded by faintly redstained DNA in counterstaining with propidium iodide (PI). In some nuclei fine dots could be detected on the thin cores. Sometimes a dot seemed to have a telomeric position, while in other cases it occured along the cores. Some nuclei exhibited only some dots but no cores. We suggest that at least a part of the dots could be the recombination nodules of early and late zygotene to early pachytene nuclei, juding by the size and chromosomal configurations of the nuclei. Only the late pachytene nuclei showed uniform staining of the cores. We could also see that spermatids and spermatozoa gave various kinds of fluorescence for anti-recA. Usually a collar surrounding the dorsal of the sperm-head was stained but sometimes an intensive staining at the tip of the head of acrosome-like material could be seen, suggesting that sperms are transporting recA-like proteins with them. The pretreatment with detergent (Triton X-100) containing buffer was not necessary for a successful staining of unfixed nuclei but led only to a background of recA positive fluorescence, which could perhaps be due to extraction of recA-positive material. Pre-extraction with 2*M* salt solution (including pre-washing with TX-100 or not) did somewhat diminish the staining but sharply stained the cores persisted in pachytene nuclei. However, when DNase digestion was included the sharply stained chromosome cores appeared completely smeared and especially with an additional 2*M* salt extraction a halo of positively stained material surrounding the bleary stained nuclei. Staining with PI did not show any red fluorescence, demonstrating that all detectable DNA had been digested. Immunooblotting of the isolated meiotic nuclei usually revealed one major (40 *kDa*) and two minor (35 and 90 *kDa*) poplypeptides.

Recent reports [3] support our findings. Moreover we have found that using unfixed the isolated meiotic nuclei we can focus on some very promising and relevant structures.

References

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